Pheochromocytoma and paraganglioma: Clinical feature based disease probability in relation to catecholamine biochemistry and reason for disease suspicion

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Abstract: OBJECTIVE Hypertension and symptoms of catecholamine excess are features of pheochromocytomas and paragangliomas (PPGLs). This prospective observational cohort study assessed whether differences in presenting features in patients tested for PPGLs might assist establishing likelihood of disease. DESIGN AND METHODS Patients were tested for PPGLs because of signs and symptoms, an incidental mass on imaging or routine surveillance due to previous history or hereditary risk. Patients with (n=245) compared to without (n=1820) PPGLs were identified on follow-up. Differences in presenting features were then examined to assess probability of disease and relationships to catecholamine excess. RESULTS Hyperhidrosis, palpitations, pallor, tremor and nausea were 30-90% more prevalent (P<0.001) among patients with than without PPGLs, whereas headache, flushing and other symptoms showed little or no differences. Although heart rates were higher (P<0.0001) in patients with than without PPGLs, blood pressures were not higher and were positively correlated to body mass index (BMI), which was lower (P<0.0001) in patients with than without PPGLs. From these differences in clinical features, a score system was established that indicated a 5.8-fold higher probability of PPGLs in patients with high than low scores. Higher scores among patients with PPGLs were associated, independently of tumor size, with higher biochemical indices of catecholamine excess. CONCLUSIONS This study identifies a complex of five signs and symptoms combined with lower BMI and elevated heart rate as key features in patients with PPGLs. Prevalences of these features, which reflect variable tumoral catecholamine production, may be used to triage patients according to likelihood of disease.

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Pheochromocytoma and paraganglioma: Clinical feature based disease probability in relation to catecholamine biochemistry and reason for disease suspicion

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Abbreviations: PPGL, pheochromocytoma and paraganglioma; BP, blood pressure; BMI, body masss index; eCRF, electronic case report form; SOP, standard operating procedure; ROC, receiver-operating characteristic; AUC, area under curve.

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Abstract

Objective: Hypertension and symptoms of catecholamine excess are features of pheochromocytomas and paragangliomas (PPGLs). This prospective observational cohort study assessed whether differences in presenting features in patients tested for PPGLs might assist establishing likelihood of disease.

Design and methods: Patients were tested for PPGLs because of signs and symptoms, an incidental mass on imaging or routine surveillance due to previous history or hereditary risk. Patients with (n=245) compared to without (n=1820) PPGLs were identified on follow-up. Differences in presenting features were then examined to assess probability of disease and relationships to catecholamine excess.

Results: Hyperhidrosis, palpitations, pallor, tremor and nausea were 30-90% more prevalent ($P<0.001$) among patients with than without PPGLs, whereas headache, flushing and other symptoms showed little or no differences. Although heart rates were higher ($P<0.0001$) in patients with than without PPGLs, blood pressures were not higher and were positively correlated to body mass index (BMI), which was lower ($P<0.0001$) in patients with than without PPGLs. From these differences in clinical features, a score system was established that indicated a 5.8-fold higher probability of PPGLs in patients with high than low scores. Higher scores among patients with PPGLs were associated, independently of tumor size, with higher biochemical indices of catecholamine excess.

Conclusions: This study identifies a complex of five signs and symptoms combined with lower BMI and elevated heart rate as key features in patients with PPGLs. Prevalences of these features, which reflect variable tumoral catecholamine production, may be used to triage patients according to likelihood of disease.

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Introduction

Clinical relevance of pheochromocytomas and paragangliomas (PPGLs) relates to their capacity to secrete catecholamines excessively, thereby producing cardiovascular complications and life-threatening events (1-3). Clinical presentation is highly heterogeneous ranging from normotensive and asymptomatic cases to patients with sustained hypertension and others with dramatic swings in blood pressure (BP) accompanied by diverse signs and symptoms during BP highs, even involving hypertensive crises (4-7). Headaches, hyperhidrosis and palpitations dominate the clinical presentation of PPGLs, particularly during paroxysmal spells (4, 8, 9). Other features include pallor, panic or anxiety, chest pain, nausea, vomiting, weakness, fatigue, weight loss and constipation (4).

Several factors contribute to the variable clinical manifestations of PPGLs including the type and pattern of catecholamine secretion, sensitivity of impacted adrenoceptors, co-secretion of peptides and tumor size. Patients may also present with a host of comorbid complications (e.g., diabetes, cardiomyopathy, stroke, unexplained shock) that can confuse the clinical picture. Due to these non-specific manifestations the differential diagnosis is often difficult; consequently the tumor has been referred to as the "great mimic" or a "clinical chameleon" (4, 10).

Further complicating diagnosis, there are now changes in how PPGLs are coming to attention. Whereas previously affected patients were mainly diagnosed based on signs and symptoms, today the tumors are increasingly found incidentally as part of imaging studies for purposes unrelated to suspicion of PPGL (6, 11, 12). Due to recognition of an increasing number of pathogenic mutations and a high rate of disease recurrence, PPGLs are now being found during surveillance screening in patients with a genetic predisposition or past history of disease (11). Such patients can be asymptomatic and normotensive (13).

The changing modes of discovery of today's patients with PPGLs bring to question whether presenting features classically attributed to the tumors remain relevant to likelihood of disease. Furthermore, although the literature is replete with reports on the clinical presentation of patients with PPGLs, there are few comparisons to patients without PPGLs or large sized studies of patients with and without PPGLs according to today's different modes of disease discovery (14).

The objective of the present study was to identify clinical features of patients tested for PPGLs that might be used to better distinguish those with and without disease and whether such features might also relate to differences in tumoral catecholamine production. This objective was met through a prospective screening study of over 2,000 patients tested for PPGLs including 245 patients in whom disease was subsequently confirmed (15). Cardiovascular parameters and other clinical features of tumors were compared to determine those most useful according to whether suspicion of disease was based on periodic surveillance associated with hereditary risk or past history of disease, findings of an incidentaloma or clinical features of catecholamine excess. Examination of presenting features in patients with PPGLs took into account tumor size and biochemical indices of catecholamine excess.

Materials and Methods

Subjects

A total of 2,291 patients were prospectively recruited into this observational cohort study under a multicenter clinical protocol (Prospective Monoamine-producing Tumor study - PMT) and according to standard-operating procedures (SOPs) available online (https://pmt-study.pressor.org) with further details outlined elsewhere (15) and in the supplemental methods. All patients enrolled into the study were recruited according to four study entry criteria: 1. signs and symptoms of presumed catecholamine excess; 2. incidental finding of an abdominal mass requiring further investigation for a PPGL; 3. screening due to previous history of PPGL; and 4. hereditary risk of PPGL associated with a mutation of a tumor susceptibility gene.

Registration of patients was facilitated using electronic case report forms (eCRFs) according to a coding system enabling collection and storage of pseudoanonymized patient data as detailed in the supplemental methods. The PMT study protocol was approved by University Hospital Dresden Ethics Committee (EC), the Radboud University Medical Center EC, the University Hospital Würzburg EC, the University Medical Center Schleswig-Holstein EC, the Klinikum der Ludwig-Maximilians-Universität München EC and the Institute of Cardiology EC at Warsaw. All subjects provided written informed consent before study inclusion.

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Clinical Annotations

Demographic and clinical data (i.e., sex, age, weight, height, systolic and diastolic BP, heart rate, signs, symptoms and antihypertensive medications) were acquired according to SOPs at study entry. Patients were evaluated according to a questionnaire to establish occurrence of signs and symptoms over 30 days before study entry. Signs and symptoms evaluated included headache, hyperhidrosis, palpitations, tremor, pallor, flushing, panic/anxiety, nausea/vomiting, weakness and constipation.

Presence of hypertension was established by systolic BP above or equal to 140 mmHg or diastolic BP above or equal to 90 mmHg. Hypertension was also defined in patients with a history of high BP controlled by antihypertensive medications. History of paroxysmal hypertension was assessed from patient notes and interviews.

Mean tumor diameters were calculated from tumor volumes, which were estimated using the formula, $V = \pi/6$ (x*y*z), where x, y, z are the three dimensions. For patients with multifocal primary tumors, tumor volumes were summed. All three dimensions were recorded in 199 (81.2%) patients, whereas two diameters in 19 (7.8%) patients were reported with the 3rd dimension estimated as the average of the two reported dimensions. For four patients (1.6%) only one dimension was reported and no information on tumor size was available in 23 (9.4%) patients, these representing mainly cases of metastatic disease that precluded determination of tumor volume.

Biochemical tests of catecholamine excess

Biochemical testing at entry of patients into the protocol included mass spectrometric-based measurements of catecholamine metabolites (metanephrine, normetanephrine, methoxytyramine) in their free unconjugated form in plasma and 24-hour urine collections, the latter also allowing measurements of catecholamines (epinephrine and norepinephrine). All details including blood and urine collections, reference intervals and designations of tumors as having an epinephrine-producing adrenergic phenotype or a non-adrenergic phenotype are described elsewhere (15-17).

Data Analyses

Statistical analyses utilized the JMP software package (SAS Institute Inc, Cary, NC). The Kruskal-Wallis and the Steel Dwass all-pairs tests were used for non-parametric comparisons of continuous data involving multiple groups. Chi- square and Fisher's exact tests were used to compare frequencies of clinical features and antihypertensive use, with a Bonferroni correction for non-continuous data. Clinical features that showed consistent differences between patients with and without PPGLs were examined in scoring systems to assess probabilities of disease and relationships of scores with tumor size and catecholamine biochemistry. Probabilities of having disease based on the scoring system were calculated according to the basis of disease suspicion (i.e., study inclusion group). Impacts of the presence or absence of PPGLs, study inclusion group, sex, age and body mass index (BMI) on cardiovascular variables and symptom or clinical feature scores were examined by least square multivariate analyses. Relationships of biochemical indices of catecholamine excess with tumor size, study inclusion group and clinical feature scores were similarly examined by multivariate analyses, carried out after normalization of biochemical and tumor size data by logarithmic transformation. Data are displayed as means and 95% confidence intervals where relevant.

Results

Patient cohorts: confirmation and exclusion of PPGLs

After biochemical testing, patients underwent follow-up to confirm or exclude PPGLs; thereafter, the final study population, after exclusions, included 245 patients with PPGLs and 1820 without tumors (supplemental methods). Due to sample size limitations patients enrolled according to a previous history of PPGLs or hereditary risk were combined into a single group according to the need for periodic surveillance (surveillance group). Among all patients, most (62.9±2.1%) were included based on clinical suspicion due to signs and symptoms compared to smaller proportions included due to an incidentaloma (23.3±1.8%) or as part of surveillance (13.8±1.5%) according to past history of disease or hereditary risk (Table 1). Although there were no significant differences in distributions of sexes, patients with PPGLs were overall younger ($P<0.05$) than those without PPGLs, this largely reflecting more advanced age in patients of the incidentaloma group who did not have PPGLs.

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Cardiovascular variables, body mass index

BMI was lower (P<0.0001) among patients with than without PPGLs, a difference that was retained (P<0.01) in each of the three study inclusion groups (Table 1). By multivariate analysis, differences in BMI according to presence or absence of PPGLs and study inclusion group were independent of age and sex. Systolic and diastolic BPs were lower (P<0.001) in patients with than without PPGLs, but these differences disappeared after multivariate correction for age, sex, BMI and study inclusion group (Table 2). In contrast, heart rate was on average 6.5 bpm higher (P<0.0001) in patients with than without PPGLs, a difference that retained significance (P<0.001) in all three patient groups (Table 1) and after multivariate correction for age, sex, BMI and study inclusion group (Table 2).

The prevalence of hypertension at study entry was relatively high among all study inclusion groups, ranging respectively in patients with and without PPGLs from 71±11% and 58±7% in the surveillance group to 78±4% and 80±8% in the incidentaloma group and 93±2% to 95±5% in the signs and symptoms group (Table 1). As expected, patients of the latter group had a higher (P<0.001) prevalence of hypertension than other groups. Although the presence of hypertension in patients with and without PPGLs at study entry did not differ, the prevalence of established hypertension before study entry was higher (P<0.0001) in patients without than with PPGLs (Supplemental table 1).

Associated with the overall higher prevalence of established hypertension in patients without than with PPGLs, there was a higher (P<0.0001) prescribed use of antihypertensives at study entry for patients without compared to with PPGLs (Supplemental table 1). As outlined in the supplemental methods section differences in heart rate and blood pressure in patients with and without PPGLs remained independent of differences in antihypertensive therapy. Reported prevalences of episodic or paroxysmal hypertension did not differ significantly between patients with and without PPGLs (35.8±6% vs 40.5±2%).

Signs and symptoms of catecholamine excess

Among examined signs and symptoms some such as headaches, flushing and panic/anxiety showed no differences in prevalence between patients with and without PPGLs, including patients tested due signs and symptoms of presumed catecholamine excess (Table 3). In contrast, hyperhidrosis, tremor,

pallor and nausea showed 66% to 102% higher (P<0.0001) prevalences in patients with than without PPGLs, including consistently higher (P<0.05) prevalences for the first three symptoms for all three study inclusion groups.

Overall the prevalence of palpitations was only 28% higher (P=0.0009) in patients with than without PPGLs (Table 3). The higher prevalence was independent of prescribed use of beta-adrenergic blockers and differences in heart rate (supplemental results). Although palpitations had the highest reported prevalence (65%) in patients with PPGLs of the signs and symptoms group, palpitations were also reported by 44% of patients without PPGLs indicating non-specificity of this symptom. Muscle weakness and constipation additionally showed 23% to 60% higher (P<0.02) prevalences in patients with than without PPGLs, but this did not reach significance for incidentaloma and surveillance groups. Although the classic triad of headaches, hyperhidrosis and palpitations had a relatively high specificity (90%), only 19% of patients with PPGLs presented with this triad.

Score system for signs and symptoms and other features of catecholamine excess

Using the five signs and symptoms that showed significant and consistent differences between patients with and without a PPGL for at least two of the three study inclusion groups, a signs and symptoms score system was established from zero to five according to the sum of points for each sign (pallor) and symptom (hyperhidrosis, palpitations, tremor and nausea). According to receiver-operating characteristic curves the combination of this symptom score with HR and BMI yielded higher (P<0.0001) areas under the curve than any feature alone (Supplemental figure 1).

Multivariate analysis accounting for age, sex, BMI, presence or absence of PPGLs and study inclusion group established, as expected, higher (P<0.0001) scores in patients with than without PPGLs and higher (P<0.0001) scores in patients tested for PPGLs in the signs and symptoms group than other groups (Table 2). Sex and BMI, but not age, were other factors associated with differences (P<0.0001) in signs and symptoms score, with female sex showing a particularly strong impact towards higher scores.

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Among the 669 patients with a BMI less than 25 kg/m^2, 20.0% had PPGLs compared to 12.0% for all patients, indicating a 67% higher probability of tumors. In contrast, among the 584 patients who were categorized as obese (i.e., BMI > 30 kg/m^2) only 4.8% had PPGLs indicating a 60% lower probability of PPGLs. Additionally, as selected from comparisons of interquartiles, 26.2% of patients with PPGLs had a heart rate of 85 bpm or more compared to 12.2% of patients without PPGLs. Because of these differences, the basic signs and symptoms score was adapted with inclusion of BMI and heart rate. For this the scoring system was amended with a negative point for obese patients and an extra point for patients with a BMI less than 25 kg/m^2. A single point was added for heart rates equal to or above 85 bpm, providing a maximal total score of 7 points. Low clinical feature scores of -1 to 0 were present in 35.0% of the study population, but only 4.2% of low scoring patients had PPGLs. In contrast, high scores of 3 or more were present in 21.2% of patients among whom 24.5% had PPGLs.

As shown in Table 4 the likelihood of having a PPGL among patients with a high clinical feature score was 5.8-fold higher than for patients with a low score, but this varied from a 3.0-fold higher likelihood in the surveillance group to 7.5- and 11.5-fold higher respective likelihoods in incidentaloma and signs and symptoms groups.

Relationships of catecholamine biochemistry with clinical feature scores and tumor size

Plasma and urinary free metanephrines as well urinary catecholamines showed expected differences among patients with and without PPGLs according to reasons for biochemical tests (Supplemental table 2). Patients with PPGLs showed differences in urinary catecholamines as well as urinary and plasma free metanephrines according to designation in the three clinical feature score groups (Figure 1). Specifically all three biochemical indices of catecholamine excess were higher ($P<0.02$) in patients with high clinical feature scores than those with low and medium scores. Plasma and urinary free metanephrines were also higher ($P<0.05$) in patients with medium than low scores.

In contrast to the differences in catecholamine biochemistry, mean tumor diameter did not differ among patients with low, medium or high scores (Figure 1). Tumor diameter nevertheless showed strong positive relationships with plasma metanephrines and urinary free metanephrines, but weaker relationships with urinary catecholamines (Figure 2). According to those relationships examined

according to clinical feature score group (Figure 2, panels A-C), study inclusion group (Figure 2, panels D-E), multivariate analyses (Supplemental table 3) or examination of indices of catecholamine excess relative to tumor diameter (Supplemental figure 2), it was clear that indices of catecholamine excess varied independently of tumor size according to both clinical feature score and inclusion groups. Specifically, patients with high clinical feature scores had larger increases ($P<0.005$) in indices of catecholamine excess relative to tumor size than patients with low sores. Furthermore, patients in surveillance and incidentaloma groups had smaller increases ($P<0.05$) in indices of catecholamine excess relative to tumor size than patients in the signs and symptoms group.

Patients with adrenergic epinephrine-producing tumors were older ($P<0.0001$) had higher ($P=0.0269$) basic symptom scores as well as higher ($P<0.05$) prevalences of tremor, pallor and panic/anxiety compared to patients with PPGLs that did not produce significant amounts of epinephrine (Table 5).

Discussion

This study provides unique data from comparisons of clinical features among a large group of patients screened for PPGLs and from this identifies key discriminating features for a scoring system to assess likelihood of disease. Links of discriminating features to differences in tumoral catecholamine production were identified, thereby clarifying why some patients do not present with the same features as others even when tumors are similarly sized. Unlike previous efforts the present investigation considered underlying reasons for clinical suspicion of disease and covered patients with and without PPGLs identified from the same cohort prospectively tested for disease. In this way the data provide novel and valuable information for physicians about how to assess the likelihood of a PPGL in relation to clinical suspicion based on signs and symptoms or the increasingly more common indications of an incidentaloma or surveillance based on genetic risk or past history of the tumors.

As clarified in a systematic review of the literature paired down to 36 studies reporting on the clinical presentation of patients with PPGLs (14), most reports do not provide comparative data from patients without tumors. Among the few with comparative data, most involved highly selected or limited numbers of patients (18-20). In 1981 Plouin and colleagues established that the triad of headache,

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palpitations and hyperhidrosis was present in 10 of the 11 patients (91%) with PPGLs compared to 6.5% of the 2585 patients with hypertension suggesting utility of the triad for assessing likelihood of disease (8). A subsequent report in 1984 from Black and Bursten (9) outlined a scoring system that combined signs and symptoms with biochemical test results from 60 patients with PPGLs, another 25 in whom PPGLs were excluded and 410 hypertensives. As in the study of Plouin et al, headache, hyperhidrosis and palpitations were the dominant presenting features in patients tested for PPGLs. Nevertheless, although present at higher prevalences in patients with than without PPGLs the triad also exhibited a relative high frequency among those without PPGLs illustrating its non-specific nature. Subsequent reports, although limited to patients with PPGLs, have indicated relatively lower proportions of patients presenting with the "classic triad" (21-23). This is in keeping with the findings here that the symptom triad was present in less than 25% of patients with PPGLs and at prevalences on average only 2-fold higher than in patients without PPGLs.

The aforementioned shortcomings in published reports mean that although there exists a constellation of established clinical features for catecholamine-producing tumors it remains unclear how these translate into meaningful information for triaging patients according to likelihood of disease. The present report addresses this need by defining relative prevalences of clinical features in patients with and without PPGLs. In most cases the findings are in accord with accepted views, in others they are not. For example many signs and symptoms of apparent catecholamine excess were as expected more prevalent in patients with than without PPGLs; however, others such as headache were not. While this and the relatively small differences in prevalences between the two groups supports the non-specific nature of these features, it was nevertheless a surprise that office measurements of BP and presence hypertension were not helpful in assessing likelihood of PPGLs. Another study has additionally indicated lower BP in patients with PPGLs than essential hypertensives (24). These findings are consistent with the conclusions of a meta-analysis indicating that hypertension is not important compared to other features to assess probability of PPGLs and that normotension might in fact be a more important consideration (14).

While the above findings and conclusions appear to fly in the face of accepted dogma, there is need to consider that at least for the group evaluated because of signs and symptoms these patients would be expected to have an *a priori* high prevalence of hypertension independent of the presence of PPGLs. Also important are the findings derived from multivariate analyses showing that the lower BPs in patients with than without PPGLs lost significance after correction for age, sex and specially the lower BMI in patients with than without PPGLs. Significant impacts of catecholamine-producing tumors on body weight, adiposity and energy metabolism are well documented (25-27), with differences in BMI reflecting differences in catecholamines (28). Here we show that among patients tested for PPGLs, those with a BMI lower than 25 kg/m^2 have a higher prevalence compared to a lower prevalence of PPGLs in obese patients. Thus, both heart rate and BMI, but not office BP, can provide information about likelihood of PPGLs in patients suspected with or at risk of PPGLs.

On the basis of the above findings a score system was devised that integrated information from selected signs and symptoms with BMI and heart rate. Hyperhidrosis, palpitations, tremor, pallor and nausea provided the five components of the signs and symptoms complex selected based on consistency of differences between groups with and without PPGLs. With the resulting clinical feature score system, relative probabilities of disease according to low to high scores can be combined with information on disease prevalence to refine estimates of disease probability ahead of triaging patients and biochemical testing. As detailed in the supplemental discussion such information can be useful for determining probabilities of disease after biochemical testing. Thus, for patients with high pre-test prevalences of PPGLs, such as those with incidentalomas, the presence of a high versus a low clinical feature score in combination with even small to mildly increased biochemical test results could define a difference between more rapidly advancing to surgery as opposed to further follow-up confirmatory biochemical testing or functional imaging. For other patients with low pre-test prevalences of PPGLs, low score lack of any relevant clinical features may minimize need for biochemical testing, particularly compared to patients with high clinical feature scores who are more likely to harbor a PPGL.

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Most clinical manifestations of PPGLs result from hemodynamic and metabolic actions of catecholamines secreted by the tumors. Accordingly and as expected, symptomatic patients with high clinical feature scores showed higher urinary catecholamines than those with lower scores. They also showed higher plasma and urinary free metanephrines, which reflects catecholamine vesicular store size and continuous leakage of catecholamines from those stores into the cytoplasm followed by local metabolism (29). Interestingly patients with low clinical feature scores were characterized by tumors that produced catecholamines at lower rates relative to tumor diameter than patients with higher scores. Thus, more highly concentrated stores of catecholamines in some tumors and associated higher rates of catecholamine secretion may contribute to why some patients with similarly sized PPGLs are diagnosed on the basis of signs and symptoms while others present as incidentalomas. This conclusion is consistent with other reports of lower indices of catecholamine production and secretion in patients with PPGLs presenting as incidentalomas than for other reasons (5, 12).

Other differences in disease presentation have been suggested to relate to the relative abundances at which PPGLs secrete epinephrine versus norepinephrine (30-32). In keeping with this concept, patients with epinephrine-producing adrenergic tumors were characterized by an overall higher symptom score and in particular more tremor, pallor and panic/anxiety than patients with PPGLs that did not produce epinephrine. Other findings consistent with those of previous reports include the higher ages of disease presentation for patients with adrenergic than other PPGLs (33), higher reported prevalence of signs and symptoms in females than males (34), and the expectedly higher prevalences of hypertension and biochemical indices of catecholamine excess in patients with PPGLs suspected due to signs and symptoms compared to other indications (11, 23).

While the prospective nature of patient recruitment, large sample size and inclusion of patients with and without PPGLs represent study strengths there are limitations, such as difficulties in establishing paroxysmal spells (see supplemental discussion). Despite those limitations, this study does provide unique data to better facilitate clinical interpretation of presenting features in patients with suspected PPGLs. While the limited value of office BP measurements for determining likelihood of PPGLs was a surprising finding that departs from established dogma, this may in part reflect changes in how the

tumors are nowadays detected along with a rising prevalence of obesity and associated hypertension in the general population.

Declaration of interest: The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study. Martin Fassnacht is on the editorial board of EJE. Martin Fassnacht was not involved in the review or editorial process for this paper, on which he is listed as an author.

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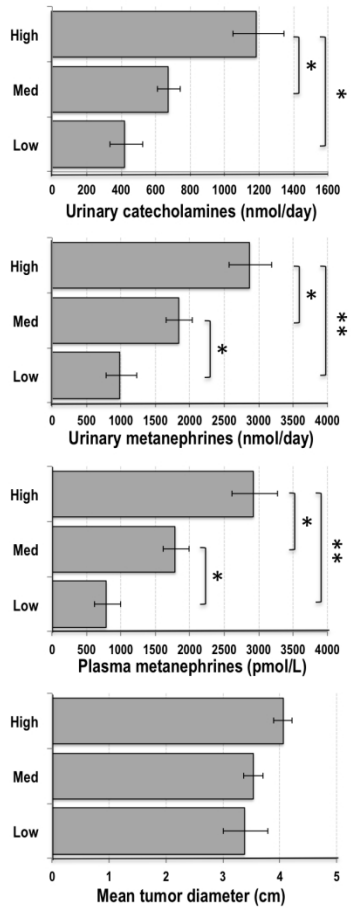
Figure legends

Figure 1. Differences in urinary catecholamines, urinary free metanephrines, plasma metanephrines and mean tumor diameter according to low, medium (Med) and high presentation scores among patients with PPGLs. Presentation scores were based on a point for the presence of each of the top five signs or symptoms that showed significant differences between patients with and with PPGLs, an additional point each for a heart rate of 85 or more or a body mass index < 25 kg/m^2 with subtraction of one point for a body mass index > 30 kg/m^2. Patients with scores of -1 or 0 were assigned to the low score group, those with scores of 1 or 2 to the medium score group and those with scores above 3 to the high score group. Data are restricted to the 90.6% of patients with PPGLs in whom measurements of mean tumor diameter were possible.

Figure 2. Relationships of mean tumor diameter with plasma free metanephrines (panels A & C), urinary free metanephrines (panels B & D) and urinary catecholamines (panels C & E) in patients with PPGLs. Relationships for panels A, B and C are separately shown for patients with high (③) and medium (□) presentation score groups compared to the low score group (③). Relationships for panels D, E and F are separately shown for patients tested due to an incidentaloma (□) or undergoing routine screening due to previous history or genetic risk (③) compared to patients tested due to signs and symptoms of catecholamine excess (③). Data are restricted to the 90.6% of patients with PPGLs in whom measurements of mean tumor diameter were possible.



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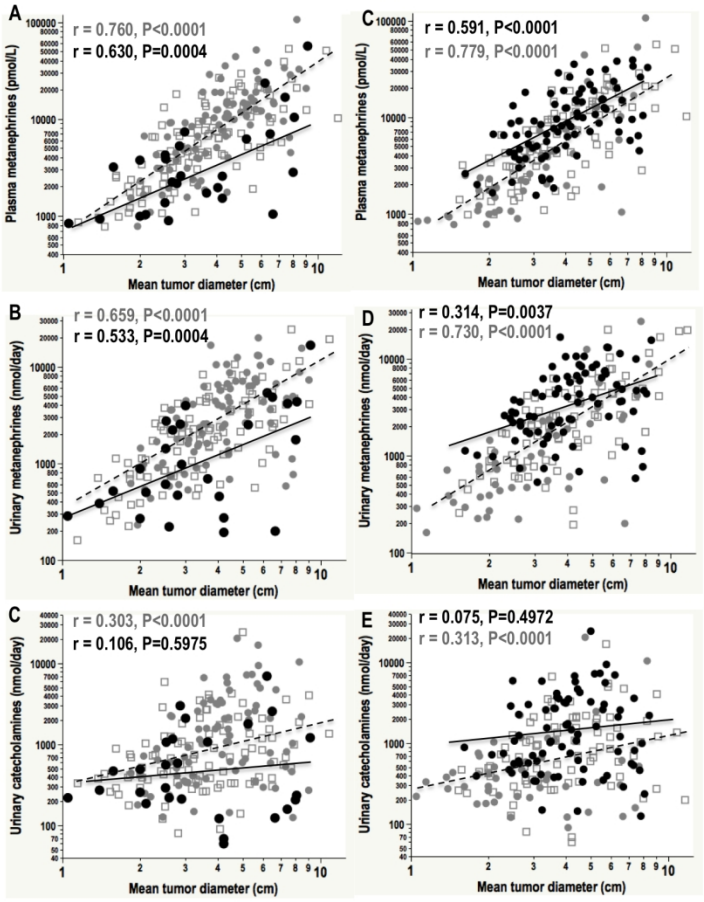

Figure 2. Relationships of mean tumor diameter with plasma free metanephrines (panels A & C), urinary free metanephrines (panels B & D) and urinary catecholamines (panels C & E). Relationships for panels A, B and C are separately shown for patients with high (●) and medium (□) presentation score groups compared to the low score group (●). Relationships for panels D, E and F are separately shown for patients tested due to an incidentaloma (□) or undergoing routine screening due to previous history or genetic risk (●) compared to patients tested due to signs and symptoms of catecholamine excess (●).

549x793mm (72 x 72 DPI)

Table 1. Demographics, body mass index, blood pressure and heart rate in patients with and without PPGLs and according to study inclusion group

	All patients	Signs & symptoms	Incidentaloma	Surveillance
Sex (% male)				
NoPPGL	49.5±2.3% (901/1820)	51.5±2.8% (621/1207)	44.9±4.9% (177/394)	47.3±6.6% (103/219)
PPGL	44.1±6.2% (108/245)	43.9±10.2% (40/91)	43.2±10.4% (38/88)	45.5±12% (30/66)
P-value	0.1176	0.192	0.813	0.8884
Age (yr)				
NoPPGL	52.1±0.7	50.1±0.8	58.8±1.2**	50.9±2.0 †
PPGL	49.9±1.9	48.8±3.2	53.1±2.8	47.1±3.7 §
P-value	0.0318	0.4043	0.0004	0.087
Body mass index (kg/m^2)				
NoPPGL	28.2±0.3	28.3±0.3	28.6±0.6	26.8±0.7**†
PPGL	25.0±0.6	25.3±0.8	24.9±1	24.8±1.1
P-value	<0.0001	<0.0001	<0.0001	0.0063
Systolic blood pressure (mmHg)				
NoPPGL	141.7±0.9	143.6±1.2	140.6±1.9	133.7±2.6**†
PPGL	135.7±2.5	139.2±4.4	136.7±4	129.8±3.9*
P-value	<0.0001	0.025	0.1134	0.1628
Diastolic blood pressure (mmHg)				
NoPPGL	86.0±0.6	86.8±0.6	85.2±1.1	82.7±1.5**§
PPGL	82.8±1.5	82.9±2.3	83.8±2.4	81.4±2.8
P-value	0.0005	0.0138	0.3878	0.2388
Heart rate (bpm)				
NoPPGL	72.1±0.5	72.3±0.6	72.7±1.0	70.3±1.4§
PPGL	78.8±1.9	80.7±2.9	78.4±3.0	76.9±2.9
P-value	<0.0001	<0.0001	0.0002	<0.0001
Hypertension at study entry ¥				
NoPPGL	85.4±1.6% (1540/1803)	93.0±1.5% (1111/1195)	77.8±4.1% (304/391)**	57.6±6.6% (125/217)**
PPGL	82.9±4.7% (203/245)	94.5±4.7% (86/91)	79.6±8.4% (70/88)**	71.2±11% (47/66)**
P-value	0.2932	0.8293	0.7768	0.0608

All data are shown as means with 95% confidence intervals. Differences between patients with (PPGL) and without tumors (noPPGL) are shown with P-values, whereas difference among the three study inclusion groups are shown according to different symbols: * P<0.05, ** P<0.001 different from patients with signs and symptoms; § P<0.05, † P<0.001 different from patients with incidentaloma. ¥ Hypertension at study entry was established according to history of hypertension. antihypertensive use or findings of systolic or diastolic blood pressure equal to or above 140 and 90 mmHg.

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Table 2. Multivariate analysis of impact of age, sex, body mass index, presence or absence of PPGL and study inclusion group on blood pressure, heart rate and symptom score

	Age	Sex	BMI	PPGL	Inclusion group
Systolic blood pressure					
F-ratio	51.3	15.1	56.1	0.7	23.4
P-value	<0.0001	<0.0001	<0.0001	0.4037	<0.0001
Impact	+ ve	M > F	+ ve	no	SS > IN > SU
Diastolic blood pressure					
F-ratio	8.0	5.0	68.4	1.7	6.6
P-value	0.0048	0.0251	<0.0001	0.1889	0.0015
Impact	+ ve	M > F	+ ve	no	SS > SU
Heart rate					
F-ratio	63.3	3.1	9.7	67.3	7.3
P-value	<0.0001	0.0799	0.0019	<0.0001	0.0007
Impact	- ve	no	+ ve	Y > N	SS & IN > SU
Basic symptom score					
F-ratio	2.3	68.3	29.0	64.5	47.5
P-value	0.1295	<0.0001	<0.0001	<0.0001	<0.0001
Impact	no	F > M	- ve	Y > N	SS > IN > SU

Abbreviations: BMI, body mass index; PPGL, pheochromocytoma and/or paraganglioma; Y, yes; N, no; SS, signs and symptoms; IN, incidentaloma; SU, surveillance due to hereditary predisposition or previous history of PPGL. Impacts for continuous data (i.e., age and BMI) are indicated as + ve or - ve for respective positive and negative relationships or as "no" where there are no relationships. Impacts for categorical data (i.e., sex, PPGL and study inclusion group) are shown according to the increase of one or two categories above others or as "no" where there is no impact.

Table 3. Signs and symptoms of catecholamine excess in patients with and without PPGLs and according to study inclusion group

	All patients	Signs & symptoms	Incidentaloma	Surveillance
Headaches				
NoPPGL	39.0±2.2% (692/1774)	44.9±2.8% (525/1170)	29.2±4.5% (113/387)**	24.9±5.7% (54/217)**
PPGL	38.3±6.1% (93/243)	46.1±10.3% (41/89)	38.6±10.1% (34/88)**	27.3±10.7% (18/66)*
P-value	0.6130	0.4558	0.0562	0.4044
Hyperhydrosis				
NoPPGL	27.6±2.1% (4891774)	29.7±2.6% (347/1170)	25.1±4.3% (97/387)	20.7±5.3% (45/217)*
PPGL	45.7±6.2% (111/243)	55.1±10.3% (49/89)	46.7±10.4% (41/88)	31.8±11.2% (21/66)*
P-value	<0.0001	<0.0001	<0.0001	0.0471
Palpitations				
NoPPGL	36.0±2.2% (639/1774)	43.9±2.8% (514/1170)	21.7±4.11% (84/387)**	18.9±5.2% 41/217)**
PPGL	46.1±6.2% (112/243)	65.2±9.9% (58/89)	45.5±10.4% (40/88)*	21.2±9.8% (14/66)**†
P-value	0.0016	<0.0001	<0.0001	0.3985
Tremor				
NoPPGL	14.6±1.6% (259/1774)	18.4±2.2% (215/1170)	8.3±2.7% (32/387 **	5.5±3.03% (12/217)**
PPGL	25.1±5.4% (61/243)	32.6±9.7% 29/89)	20.5±8.4% (20/88)	18.2±9.3% (12/66)
P-value	<0.0001	0.0015	0.0065	0.0127
Pallor				
NoPPGL	13.0±1.5% (230/1774)	15.0±2.0% (176/1170)	9.8±2.9% (38/387)*	7.4±3.4% (16/217)*
PPGL	26.2±5.5% (63/243)	37.1±10.0% (33/89)	20.5±8.4% (18/88) *	18.2±9.3% (12/66)*
P-value	<0.0001	<0.0001	0.0065	0.0127
Flushing				
No PPGL	22.9±1.9% (406/1774)	26.6±2.5% (311/1170)	16.5±3.7% (64/387)**	14.3±4.6% (31/217)**
PPGL	19.8±5.0% (48/243)	21.4±8.5% (19/89)	22.7±8.7% 20/88)	13.6±8.2% (9/66)*
P-value	0.8817	0.8881	0.1130	0.6218
Panic / anxiety				
NoPPGL	24.6±2.0% (437/1774)	31.5±2.6% (368/1170)	14.0±3.4% 54/387)**	6.9±3.3% (15/2176)**†
PPGL	24.7±5.4% (60/243)	37.1±10.0% (33/89)	19.3±8.2% (17/88)*	15.2±8.6% (10/66)*
P-value	0.5198	0.1633	0.2451	0.0394
Nausea / vomiting				
NoPPGL	11.4±1.4% (202/1774)	11.7±1.8% (137/1170)	11.1±3.1% (43/387)	10.1±4.01% (22/217)
PPGL	21.4±5.1% (52/243)	25.8±9.0% (23/89)	18.2±8.0% (16/88)	19.7±9.6% (13/66)
P-value	<0.0001	0.0004	0.0550	0.036
Weakness				
NoPPGL	34.6±2.2% (614/1774)	36.1±2.7% (422/1170)	31.8±4.6% (123/387)	31.8±6.2% (69/217)
PPGL	42.4±6.2% (103/243)	50.6±10.3% (45/89)	34.1±9.9% (30/88)*	42.4±11.9% (28/66)
P-value	0.0112	0.0049	0.3820	0.0753
Constipation				
NoPPGL	8.5±1.3% (151/1774)	8.6±1.6% (100/1170)	9.0±2.8% (35/387)	7.4±3.4% (16/217)
PPGL	13.6±4.3% (33/243)	16.9±7.7% (15/89)	10.2±6.32% (9/88)	13.6±8.2% (9/66)
P-value	0.0093	0.0116	0.4301	0.0963
Classic triad (headaches. hyperhidrosis. palpitations)				
NoPPGL	9.8±1.3% (173/1774)	11.3±1.8% (132/1170)	7.5±2.6% (29/387)	5.5±3.03% (12/217)*
PPGL	19.3±4.9% (47/243)	23.6±8.8% (21/89)	23.9±8.9% (21/88)	7.6±6.3% (5/66)*†
P-value	<0.0001	0.0013	<0.0001	0.3602

All data are shown as means with 95% confidence intervals. Differences between patients with tumors (PPGL) and without tumors (noPPGL) are shown with P-values, whereas differences between the three study inclusion groups are shown according to different symbols: *P<0.05, ** P<0.001 different from patients with signs and symptoms; † P<0.05 different from patients with incidentaloma.

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Table 4. Probabilities (%) for disease (PPGL) in all patients and according to low, medium and high clinical feature scores or according to study inclusion groups

	All patients	Signs & symptoms	Incidentaloma	Surveillance
All patients				
PPGL (YES/NO)	241/1721	90/1147	86/363	65/211
Probability	12.3±1.5%	7.3±1.4%	19.2±3.6%	23.6±5.0%
Low clinical feature score (-1 or 0)				
PPGL (YES/NO)	29/661	6/408	10/165	13/88
Probability	4.2±1.5%	1.5±1.2%	5.7±3.4%	12.9±6.5%
Medium clinical feature score (1 or 2)				
PPGL (YES/NO)	110/746	34/499	42//152	34/95
Probability	12.9±2.2%	6.4±2.1%	21.7±5.8%	26.4±7.6%
High clinical feature score (> 3)				
PPGL (YES/NO)	102/314	50/240	34/46	18/28
Probability	24.5±4.1%	17.2±4.3%	42.5±10.8%	39.1±14.1%

Probabilities are shown as means with 95% confidence intervals.

Table 5. Adrenergic phenotype* of PPGLs and disease presentation

	Adrenergic	Not adrenergic	P-value
Sex (% males)	38.3% (49/128)	50.4% (59/117)	0.0712
Age (yr)	54.1±2.7	45.3±2.4	<0.0001
BMI (kg/m2)	25.5±0.8	24.4±0.8	0.0417
SBP (mmHg)	136.7±3.7	134.5±3.1	0.8371
DBP (mmHg)	82.6±2.2	82.9±2.2	0.9383
HR (bpm)	78.5±2.6	79.2±3.0	0.9728
Headache	33.9±8.2% (43/127)	43.1±9.0% (50/116)	0.1481
Hyperhidrosis	45.7±8.7% (58/127)	45.7±9.1% (53/116)	1.0000
Palpitations	51.2±8.7% (65/127)	40.5±8.9% (47/116)	0.1220
Tremor	30.7±8.0% (39/127)	19.0±7.1% (22/116)	0.0388
Pallor	34.4±6.9% (44/127)	16.4±6.7% (19/116)	0.0013
Flushing	19.7±8.3% (25/127)	19.8±7.3% (23/116)	1.0000
Panic / anxiety	32.3±8.1% (41/127)	16.4±6.7% (19/116)	0.0046
Nausea / vomiting	26.0±7.6% (33/127)	16.4—6.7% (19/116)	0.0849
Weakness	41.0±8.6% (52/127)	44.0±9.0% (51/116)	0.6970
Constipation	14.2±6.1% (18/127)	12.9±6.1% (15/116)	0.8523
Symptom score	1.86±0.28	1.38±0.22	0.0269

All variables are shown as means with confidence intervals. *The adrenergic phenotype of PPGLs was determined from plasma concentrations of metanephrine relative to the other two metabolites as outlined in the Methods section and serves to designate PPGLs that produce epinephrine compared to other tumors that do not.

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Supplemental appendix

Pheochromocytoma and paraganglioma: Clinical feature based disease probability in relation to catecholamine biochemistry and reason for disease suspicion

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This supplemental appendix was produced in response to comments from referees to both minimize length of the manuscript and provide additional methodological details and other information not present in the first submitted version of the article. Much of that information, as provided here, is also outlined in a previously published supplemental appendix that is publically available on-line by open access (http://clinchem.aaccjnls.org/content/clinchem/suppl/2018/07/20/clinchem.2018.291369.DC1/Supplemental-appendix.pdf). Every attempt has been made to minimize repetition of the same material here.

Supplemental methods

Study rationale

The prospective monoamine-producing tumor (PMT) study was designed to address several objectives as outlined in the study protocol, which is available online (https://pmt-study.pressor.org). The primary goal of this multicenter cross-sectional cohort study was to identify new and improved disease biomarkers and establish the biochemical and molecular basis for variations in the clinical presentation of monoamine-producing tumors, particularly paragangliomas and pheochromocytomas (PPGLs). The first objective was to compare the utility of different biochemical tests to diagnose PPGLs. This objective was addressed in a related manuscript (1), available on-line (http://clinchem.aaccjnls.org/content/64/11/1646) and accompanied by a supplemental appendix (http://clinchem.aaccjnls.org/content/clinchem/suppl/2018/07/20/clinchem.2018.291369.DC1/Supplemental-appendix.pdf), which together provide background material relevant to the present manuscript. As outlined in the clinical protocol and online supplemental appendix, accrual into the PMT study was capped at 2,400 patients according to a power analysis that also required at least one patient with confirmed PPGLs for every twelve who were screened for disease.

A recognized limitation of the study design involved the 1:12 projected proportions of patients with and without PPGLs, which does not reflect the lower pretest prevalences of patients with disease commonly encountered at primary through to tertiary medical centers. The enrichment of the study population with patients with disease was, however, necessary in order to enable enrollment of a sufficient number of patients with PPGLs without overburdening participating centers with requirements for recruitment and follow-up of enrolled patients. With recognition of this limitation, it was anticipated that data would be modeled according to already established data on pre-test prevalences of PPGLs.

A secondary objective of the PMT study was to establish relationships between catecholamine-related biochemical phenotypes of PPGLs with cardiovascular, metabolic and other complications of the tumors. That objective provides the basis for the present study, as outlined in the manuscript that accompanies this particular supplemental appendix. An additional rationale for the present study was to build on the results of the already published diagnostic study (1), in which post-test probabilities of disease could be assessed from the biochemical test results, but only with knowledge of pre-test probabilities of disease. It was hypothesized that information about differences in clinical features between patients with and without PPGLs could be used to refine pre-test probabilities of PPGLs for improved translation of biochemical test results into post-test disease probabilities. This hypothesis provides the basis for stated objective of the present study to identify clinical features of patients tested for PPGLs that might be used to better distinguish patients with and without disease. The additional objective of establishing whether these features might relate to differences in catecholamine-related biochemical phenotypes is directly relevant to the secondary objective of the PMT study as outlined earlier.

Patient selection and flow through the protocol

Recruitment of patients into the PMT protocol, beginning in 2011 and ending in 2018, was carried out at six tertiary care medical centers: 1. University Hospital Carl Gustav Carus Dresden, Germany; 2. University Medical Centre Schleswig-Holstein, Lübeck, Germany; 3. University Hospital of Munich, Germany; 4. University Hospital of Würzburg, Germany; 5. Radbound University Medical Centre, Nijmegen, the Netherlands; and 6. the Institute of Cardiology, Warsaw, Poland. Patients were selected for inclusion into the protocol based on clinical suspicion or high risk of a PPGL. For inclusion based on clinical suspicion, patients had to present with signs, symptoms or clinical manifestions that could reflect catecholamine excess. Increased risk for PPGLs was based on three other criteria: 1. an

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incidentally discovered mass during imaging studies carried out for reasons unrelated to suspicion of PPGL; 2. hereditary risk of PPGLs according to syndromic presentation, family history, or an established mutation of a tumor-susceptibility gene; and 3. a previous history of a resected PPGL.

A total of 2,291 patients were recruited into the study according to above selection criteria. Among these, 2065 patients were selected for final inclusion. Recruitment was followed by biochemical screening for PPGLs and collection of patient data including measurements of height, body weight, office blood pressure, heart rate and signs and symptoms of presumed catecholamine excess. Documentation of these signs and symptoms were available in 2009 (97.3%) of the 2065 patients included in the study. Although documentation also included queries concerning frequency, duration and association with other signs and symptoms, those collected data were limited. Details of medications were also collected into electronic case report forms (eCRFs); those data were available from 1954 (94.6%) of the 2065 patients included in the study.

The results of biochemical screening were subsequently used to channel patients into different study phases. Patients with high probability of PPGLs were transferred to phase III for disease characterization (i.e. imaging and other studies). In most of these cases this was followed by surgical intervention and phase IV-B confirmation of disease by histopathology. Patients with lower probability of PPGLs, based on positive biochemical tests that were insufficiently elevated to strongly indicate a catecholamine-producing tumor, were transferred to phase II for further investigations (e.g., clonidine suppression tests, follow-up biochemical testing at later time points) to assist with confirmation or exclusion of disease. Patients in who PPGLs were indicated by follow-up investigations were then transferred to phase III and again in most of these cases thereafter to phase IV-B confirmation of disease by histopathology. In patients with clear biochemical or imaging evidence of PPGLs, but in whom tumors could not be resected, confirmation of disease was achieved by functional imaging, either by ^{123}I-iodo-metaiodobenzylguanidine scintigraphy or positron emission tomography with ^{18}F-fluorodihydroxyphenylalanine or ^{68}Ga-labeled analogues of octreotide (e.g., 68Ga-DOTATATE). Such cases included several isolated patients who refused surgical intervention, but more commonly others with metastatic disease or lesions considered inoperable. In total 245 patients with PPGL were confirmed in this way, but this total included several patients with negative biochemical test results who were only identified after long-term follow-up.

Exclusion of PPGLs could be achieved during initial investigations or interventions (e.g., negative results of clonidine tests, imaging studies or pathology reports for surgically resected adrenal tumors), in which case patients were transferred to phase IV-A. However, exclusion of PPGLs in the majority of patients, particularly those with negative results of biochemical screening tests, required follow-up. In more than 50% of all cases this involved a follow-up interval of over 2 years with exclusion based on several criteria that depended on the specific inclusion criteria and are detailed in the supplementary appendix to a previously published manuscript as outlined earlier. In this way PPGLs were excluded in 1820 patients of the 2291 recruited into the protocol. The remaining 226 patients in who PPGLs were neither confirmed (n=245) nor excluded (n=1820) were excluded from the study. Among these 226 patients there were 43 patients with head and neck paragangliomas, who were nevertheless excluded from the analysis since most of these tumors are non-functional and do not produce catecholamines or the classic signs and symptoms of PPGLs. The other patients who were excluded from the analysis included 76 in whom no samples for biochemical testing were received at the central Dresden laboratory and another 107 patients in whom either follow-up was inadequate or there were other problems: contraindicated medications or limitations associated with collections of urine or plasma specimens.

Supplemental results

History of hypertension and antihypertensive therapy at study entry

The overall lower (P<0.0001) established history of hypertension in patients with than without PPGLs (Supplemental table 1) largely reflected unrecognised hypertension among a significant proportion of patients with PPGLs who did not have an already established history of hypertension at study entry. Detection of high blood pressures in such patients was according to European Hypertension Society guidelines that define stage 1 hypertension by a systolic BP above or equal to 140 mmHg or diastolic BP above or equal to 90 mmHg.

The generally higher prescribed use of antihypertensives at study entry in patients without than with PPGLs was most apparent for diuretics, calcium channel blockers, beta-adrenergic receptor blockers, and antagonists of the renin angiotensin system. This higher prescribed use in patients without than with PPGLs presumably also reflected the higher prevalence of previously established hypertension in in the patient group without than with PPGLs. The solitary exception to the overall higher prescribed use of antihypertensives in patients without compared to with PPGLs involved alpha-adrenoceptor blockers, which were prescribed in nearly 60% more patients with than without PPGLs.

Multivariate analysis indicated that although alpha-blocker use was associated with lowered (P<0.0001) systolic and diastolic BPs, the lower BPs in patients with than without PPGLs remained significant (P<0.0001) both without inclusion of patients taking alpha-adrenoceptor blockers as well as after multivariate analyses considering patients prescribed and not prescribed alpha-adrenoceptor blockers. Similarly, although beta-blocker use was associated with a lower (P=0.0043) heart rates, the higher heart rates in patients with than without PPGLs remained independent (P<0.0001) of the 28% higher use of beta-blockers in patients without compared to with PPGLs.

Heart rate versus palpitations

Although palpitations and an elevated heart rate might be linked at times when patients experience the former symptom there was nevertheless no relationship between heart rate measured at study entry and patient reports of palpitations. Specifically, heart rate (mean±confidence intervals) did not differ significantly according to reported respective presence or absence of palpitations in either patients in whom PPGLs were confirmed (80.0±2.9 vs 77.4±2.5 beats/min) or excluded (72.5±0.9 vs 72.0±0.7 beats/min). This supports separate use of both an elevated heart rate and subjective patient reports of palpitations for evaluating likelihood of a PPGL and in the clinical feature score system described in the manuscript.

Receiver-operating characteristic curves

Receiver-operating characteristic (ROC) curves employing different features of clinical presentation to discriminate patients with and without PPGLs yielded different areas under curves (AUCs) for different combinations of features (Supplemental figure 1). Use of the five signs and symptoms that showed significant and consistent differences for at least two of the three inclusion groups yielded an identical AUC of 0.629 to that for heart rate, whereas the combination of both heart rate and the five symptoms yielded a higher (P<0.001) AUC of 0.682 compared to either the five signs and symptoms or heart rate alone. AUCs increased further to 0.759 (P<0.0001) with the combination of signs and symptoms, heart rate and BMI.

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As expected plasma normetanephrine, metanephrine and methoxytyramine as well as urinary free normetanephrine, metanephrine, norepinephrine and epinephrine were higher (P<0.0001) in patients with than without PPGLs (Supplemental table 2). Apart from urinary epinephrine these differences remained significant (P<0.005) across all three patient groups. For urinary epinephrine there was no difference between patients with and without PPGLs for the surveillance group; this likely reflects the mainly noradrenergic rather than adrenergic phenotypes of PPGLs in patients screened according to hereditary risk as well as the higher risk of recurrence in patients with primary tumors that do not produce epinephrine.

Patients with PPGLs in the surveillance group had lower (P<0.05) plasma concentrations and urinary outputs of free normetanephrine and metanephrine than either patients in groups with signs and symptoms or incidentalomas (Supplemental table 2). Urinary norepinephrine and epinephrine were also lower (P<0.001) in the surveillance than the signs and symptoms group, whereas for the incidentaloma group the difference was only significant (P<0.001) for epinephrine.

Among patients without PPGLs there were some isolated smaller differences with a higher (P<0.05) plasma concentrations of normetanephrine in the incidentaloma than signs and symptoms and surveillance groups. These differences likely reflected the more advanced age of patients in the incidentaloma group and the established relationship of plasma normetanephrine with age. In contrast, plasma and urinary free metanephrine as well as urinary epinephrine were lower (P<0.05) in the surveillance than the signs and symptoms group. This difference likely reflected the presence of patients in the surveillance group who had undergone adrenalectomies for previous tumors with subsequent impact on adrenal epinephrine production and plasma concentrations and urinary outputs of metanephrines.

Multivariate analyses of catecholamine biochemistry, tumor diameter and clinical features

Mean tumor diameter showed strong positive relationships with plasma concentrations of metanephrines (r=0.735, P<0.0001), urinary free metanephrines (r=0.640, P<0.0001), but weaker relationships (r=0.287, P<0.0001) with urinary catecholamines. With multivariate analysis, accounting for tumor diameter as well as study inclusion and clinical feature score groups, the positive relationships of tumor diameter with plasma and urinary free metanephrines remained highly significant (Supplemental table 3). Patients of the signs and symptoms group also had higher (P<0.01) plasma and urinary free metanephrines than those of the incidentaloma and surveillance groups. Similarly patients of the higher and medium clinical feature score groups had higher plasma and urinary free metanephrines than those of the low score group. Similar though weaker relationships and differences were observed for urinary catecholamines, which were higher (P<0.05) in patients of high than low score groups as well as patients of the signs and symptoms group than those of the surveillance group.

Since the above differences among patient groups suggest that production of metanephrines and secretion of catecholamines is related to factors besides tumor size, this was further examined by comparisons of relationships of tumor diameter with indices of catecholamine excess in patients with different clinical feature scores (Figure 2, panels A-C) and different study inclusion groups (Figure 2, panels D-E). These relationships and additional analyses (Supplemental figure 2, panels A-C) established that patients with low presentation scores were characterized by smaller (P<0.01) increases, relative to tumor diameter, in plasma metanephrines, urinary free metanephrines and urinary catecholamines than patients with higher scores. Similarly, patients of the signs and symptoms group were characterized by larger (P<0.05) increases, relative to tumor size, in plasma metanephrines, urinary free metanephrines and urinary catecholamines than patients of incidentaloma and surveillance groups (Supplemental figure 2, panels D-F).

Supplemental discussion

Translation of pre-test prevalences and probabilities to post-test probabilities of PPGLs

As we have shown elsewhere (1, 2), post-test probabilities of disease can be determined from pre-test probabilities using patterns and extents of increases in biochemical test results above cut-offs. Pre-test probabilities can be further refined using the score system described here. Thus, among unselected patients tested for PPGLs about 0.8% have a PPGL (3). This is up to 2-fold higher than prevalences in unselected hypertensives (4, 5), a group in which screening is not commonly considered nor recommended unless there are other features present that raise likelihood of a catecholamine-producing tumor. At the pre-test prevalence of 0.8%, and assuming appropriate pre-analytics and reference intervals, a single test result for plasma free metanephrines just above upper cut-offs of reference interval translates to a 14% post-test probability of disease whereas a test result 50% above cut-offs translates to a 59% probability (Supplemental figure 3).

From the data of the present report in table 4, the pretest probability for a patient tested due to signs and symptoms of presumed catecholamine excess, but who also presents with a high clinical feature score, more than doubles (17.2/7.3) to 2.4% so the post-test probabilities increase respectively from 14% to 26% and from 59% to 87% (Supplemental figure 3, panel A). In contrast for a patient with a low clinical feature score the post-test probability is decreased by nearly 80% ([7.3-1.5]/7.3) to only 0.16%, bringing such patients to a level of pre-test disease probability that makes the value of biochemical testing questionable. At this this low pre-test probability of disease the post-test probability of a tumor would be only 3% with a test result for a single increase in plasma free normetanephrine or metanephrine just above the upper cut-offs of reference intervals.

For patients with incidentalomas in whom overall pre-test prevalences of PPGLs reach 5% (6), low clinical feature scores decrease pre-test probabilities by 70% ([19.2-5.7/5.7) to 1.5%, whereas high clinical feature scores more than double (42.5/19.2) pre-test probabilities to 11%. For a plasma normetanephrine just above upper cut-offs this translates to post-test probabilities of 23% versus 71% for patients with respective low and high clinical feature scores. Other useful information about probabilities of PPGLs among patients with incidentalomas can be derived from computed tomography imaging characteristics (7); adrenal masses presenting on unenhanced computed tomography with Hounsfield units <10 are much less likely to be pheochromocytomas compared to those with higher values. Also, although patients with PPGLs discovered as incidentalomas tend to be older than others with PPGLs (8, 9), as we show here such patients are significantly younger than those with other incidental masses. Thus, age is another factor to consider when assessing the probability of a PPGL among patients with incidental abdominal masses.

Study strengths and limitations

The present study has some strengths, but also limitations. Strengths include the large numbers of patients with and without PPGLs, all of whom were selected into the study due to clinical suspicion or risk of PPGLs. The prospective nature of the study and follow-up of patients to both exclude and confirm PPGLs among patients who escaped attention after initial screening represents another strength and a design feature rarely employed in other studies of this nature.

Among study limitations, first and foremost assessments of paroxysmal hypertension were from patient histories and were found not to differ between patients with and without PPGLs. These assessments, without consistent 24-hr ambulatory blood pressure monitoring records, are questionable. Likewise, although questionnaires about signs and symptoms included queries about frequency, duration and

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associations with other signs and symptoms, those data were not collected consistently enough for reliable interpretation. As shown previously (10), increased blood pressure along with signs and symptoms of PPGLs often occur in paroxysmal spells that might better discriminate patients with and without PPGLs. The present study did not adequately address this possibility. Another limitation was that the questionnaire about signs and symptoms did not include all possible signs and symptoms, such as blurred vision. It could also have been useful to have included a third group of subjects without any known hypertension, clinical suspicion or risk for PPGLs to establish relative frequencies of signs and symptoms or presence of hypertension among the general population for additional comparative purposes. Nevertheless, such information and comparisons to patients with PPGLs are also already available.

The design of the present study was unique in employing comparisons among patients in whom disease was confirmed or excluded after selection into the study according to entry criteria of clinical suspicion of or risk for disease. An associated limitation of the study, recognized when first designed, relates to the enrichment of the study population with patients with PPGLs well above usual pre-test prevalences of disease. This was a necessity in order to avoid overburdening participating centers with requirements for recruitment and follow-up of enrolled patients. It nevertheless could have resulted in some selection bias. To address this limitation and further confirm the present data another prospective study would be required involving at least a 10-fold larger study population.

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Supplemental figure 1: ROC curves for discrimination of patients with and without PPGLs. ROC curves for the five symptom complex (Sym), heart rate (HR) and the combination of Sym and HR are shown in panel A, whereas ROC curves for body mass index (BMI), the combination of HR and BMI with and without the five symptom complex are shown in panel B.

Supplemental figure 2: Plasma concentrations of metanephrines (A, D), urinary outputs of metanephrines (B, E) and urinary outputs of catecholamines (C, F) as a function of tumor diameter (TD) in patients with low, medium (MED) and high clinical feature scores (A, B, C) or according to study inclusion criteria (D, E, F). Statistical significance was established by Dunnett's post-hoc tests, which for clinical feature score group comparisons used patients in the low score group as the comparator, whereas for study inclusion group comparisons used the signs and symptoms (S&S) group as the comparator.

Supplemental figure 3: Relationships of pre-test versus post-test probability a PPGL according to results for a single elevation of plasma free normetanephrine or metanephrine just above the upper cut-offs (1 x UC, green curves), 50% above upper cut-offs (1.5 x UC, blue curves) or 100% above upper cut-offs (2 x UC, red curves). The two panels serve to illustrate differences in pre- to post-test probabilities among patients with low compared to high clinical feature scores. Panel A illustrates changes in probabilities in a patient tested due to signs and symptoms at a 0.8% pre-test prevalence of disease, whereas panel B illustrates changes in probabilities in a patient tested due to the finding of an incidentaloma at a 5% pre-test prevalence of disease. Relationships are derived from previously published data (1) from the same group of patients that is also available in the public domain through open access.

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Supplemental table 1. Established history of hypertension and antihypertensive use at study entry in patients with and without PPGLs and according to study inclusion group

	All patients	Signs & symptoms	Incidentaloma	Surveillance
Established history of hypertension ¥				
NoPPGL	82.2±1.8% (1471/1789)	91.1±1.6% (1080/1185)	70.4±4.5% (274/389) **	54.4±6.6% (117/215) **†
PPGL	68.6±5.8% (168/245)	86.8±6.9% (79/91)	55.7±10.3% (49/88) **	60.6±11.7% (40/66)**
P-value	<0.0001	0.1844	0.0112	0.3983
Diuretics				
NoPPGL	38.2±2.3% (653/1709)	45.8±2.9% (518/1132)	27.0±4.4% (103/381) **	16.3±5.1% (32/196) **§
PPGL	13.9±4.3% (34/245)	17.6±7.8% (16/91)	12.5±6.9% (11/88)	10.6±7.4% (7/66)
P-value	<0.0001	<0.0001	0.0036	0.32
Calcium channel blockers				
NoPPGL	47.7±2.3% (815/1709)	58.7±2.8% (642/1132)	36.8±4.8% (140/381) **	16.8±5.2% (33/196) **†
PPGL	26.2±5.5% (64/245)	31.9±9.5% (29/91)	23.9±8.9% (21/88)	21.2±9.8% (14/66)
P-value	<0.0001	<0.0001	0.0247	0.4593
Beta adrenoceptor-blockers				
NoPPGL	50.1±2.3% (868/1709)	58.4±2.8% (661/1132)	38.6±4.8% (147/381) *	30.6±6.4% (60/196) **
PPGL	39.2±6.1% (96/245)	45.1±10.2% (41/91)	35.2±9.9% (31/88)	36.4±11.6% (24/66)
P-value	0.0008	0.0152	0.6264	0.4461
Alpha adrenoceptor-blockers				
NoPPGL	18.4±1.8% (315/1709)	23.5±2.4% (266/1132)	11.3±3.1% (43/381) **	3.1±2.4% (6/196) **†
PPGL	29.4±5.7% (72/245)	37.4±9.9% (34/91)	25.3±9.0% (21/88) *	25.8±10.5% (17/66)
P-value	<0.0001	0.005	0.0033	<0.0001
Renin-angiotensin system antagonists				
NoPPGL	59.4±2.3% (1015/1709)	66.9±2.7% (758/1132)	52.0±5.0% (198/301) **	30.1±6.4% (59/196) *††
PPGL	35.5±5.9% (87/245)	42.9±10.1% (39/91)	40.2±10.2% (35/88)	19.7±9.6% (13/66) *§
P-value	<0.0001	<0.0001	0.0571	0.1128
Alpha$_2$ adrenoceptor agonists				
NoPPGL	6.6±1.1% (113/1709)	8.1±1.5% (92/1131)	5.0±2.1% (19/381)	1.0±1.3% (2/196) **§
PPGL	2.5±1.9% (6/245)	1.1±2.1% (1/91)	1.1±2.1% (1/88)	6.1±5.7% (4/66)
P-value	0.0094	0.0116	0.1443	0.0368
Mineralocorticoid receptor antagonists				
NoPPGL	7.6±1.2% (129/1709)	8.1±1.5% (92/1131)	9.0±2.8% (34/380)	1.5±1.7% (3/196) *§
PPGL	4.4±2.5% (11/245)	6.6±5.1% (6/91)	3.8±3.9% (4/88)	1.5±2.9% (1/66)
P-value	0.1263	0.6436	0.2002	1.0000
Number of antihypertensives				
NoPPGL	2.3±0.08	2.7±0.09	1.8±0.16**	1.0±0.16**†
PPGL	1.5±0.16	1.8±0.28	1.4±0.25	1.3±0.28*
P-value	<0.0001	<0.0001	0.0805	0.0742

All data are shown as means with 95% confidence intervals. Differences between patients with (PPGL) and without tumors (noPPGL) are shown with P-values, whereas difference among the three study inclusion groups are shown according to different symbols: *P<0.05. ** P<0.001 different from patients with signs and symptoms; § P<0.05. † P<0.001 different from patients with incidentaloma. ¥Establishjed history of hypertension was determined according to history of hypertension and use of antihypertensives and differs from the data in table 1 hypertension was also established by measurements of blood pressure.

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Supplemental table 2. Plasma and urinary catecholamine metabolites and urinary catecholamines patients with and without PPGLs and according to the basis for disease suspicion

	All patients	Signs & symptoms	Incidentaloma	Routine screening
Plasma tests N (No PPGL / PPGL)				
	1796 / 243	1207 / 91	395 / 87	218 / 66
Plasma free normetanephrine (pmol/L)				
No PPGL	366 (260-511)	351 (246-484)	418 (309-588)**	383 (282-525)§
PPGL	3730 (1666-9765)	5853 (2646-11645)	3469 (1764-10770)	2101 (914-5937) **§
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Plasma free metanephrine (pmol/L)				
No PPGL	152 (107-208)	154 (114-209)	154 (99-216)	129 (86-193)**§
PPGL	644 (173-2496)	1311 (240-3662)	862 (201-2481)	188 (110-816)**†
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Plasma free methoxytyramine (pmol/L)				
No PPGL	30 (21-42))	28 (20-40)	32 (22-44)**	30 (22-44)
PPGL	84 (48-178)	103 (55-166)	83 (47-167)	69 (41-258)
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Urine tests N (No PPGL / PPGL)				
	1756 / 225	1173 / 82	376 / 82	207 / 61
Urinary free normetanephrine (nmol/day)				
No PPGL	120 (88-168)	118 (85-166)	124 (88-175)	119 (91-165)
PPGL	1261 (525-3010)	2043 (839-3925)	1297 (543-3676)	647 (288-1591)**§
P-value	<0.0001	0.0022	<0.0001	<0.0001
Urinary free metanephrine (nmol/day)				
No PPGL	80 (54-120)	87 (57-122)	70 (47-112)**	72 (44-119)*
PPGL	333 (91-1522)	805 (145-2107)	393 (94-1685)	98 (46-420)**†
P-value	<0.0001	<0.0001	<0.0001	0.0177
Urinary free norepinephrine (nmol/day)				
No PPGL	150 (101-226)	147 (98-229)	152 (104-213)	161 (114-238)
PPGL	498 (233-1288)	617 (98-229)	520 (237-1260)	309 (187-670)**
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Urinary free epinephrine (nmol/day)				
No PPGL	21 (12-33)	22 (13-36)	17 (11-29)**	19 (11-28)**
PPGL	73 (19-341)	179 (43-476)	87 (25-362)	18 (9-39)**†
P-value	<0.0001	<0.0001	<0.0001	0.5082

Data for plasma concentrations and urinary outputs are shown as medians and interquartiles in parentheses. Differences between patients with tumors (PPGL) and without tumors (no PPGL) are shown with P-values, whereas differences between the three study inclusion groups are shown according to different symbols:*P<0.01, ** P<0.001 different from patients with signs and symptoms; § P<0.05, † P<0.001 different from patients with incidentaloma.

Supplemental table 3. Multivariate analysis of impact of tumor diameter, study inclusion group and clinical feature score group in relation to catecholamine-related biochemistry in patients with PPGLs

	Tumor diameter	Study inclusion group	Score group
Plasma metanephrines			
F-ratio	214.0	6.6	6.3
P-value	<0.0001	0.0017	0.0023
Impact	+ ve	SS > SU	H & M > L
Urine free metanephrines			
F-ratio	104.4	9.9	4.8
P-value	<0.0001	<0.0001	0.0089
Impact	+ ve	SS > IN & SU	H & M > L
Urine catecholamines			
F-ratio	9.6	6.9	3.1
P-value	0.0022	0.0013	0.0470
Impact	+ ve	SS > SU	H > L

Biochemical indices of catecholamine excess were calculated as summed totals of individual amines.Tumor diameters were calculated as mean diameter (cm). Study inclusion group was based on whether testing was carried out due to signs and symptoms (SS), an incidentaloma (IN) or as part of surveillance (SU) due to hereditary predisposition or previous history of PPGL. Clinical feature score groups were divided into low (L), medium (M) and high (H) groups according to the scoring system outlined in the manuscript text.

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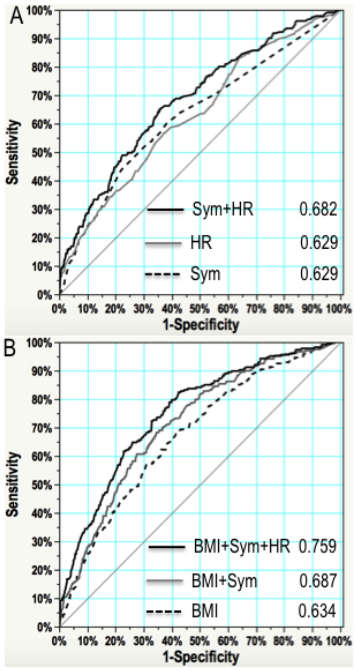

Supplemental Figure 1 / ROC curves for discrimination of patients with and without PPGLs. ROC curves for the five symptom complex (Sym), heart rate (HR) and the combination of Sym and HR are shown in panel A, whereas ROC curves for body mass index (BMI), the combination of HR and BMI with and without the five symptom complex are shown in panel B.

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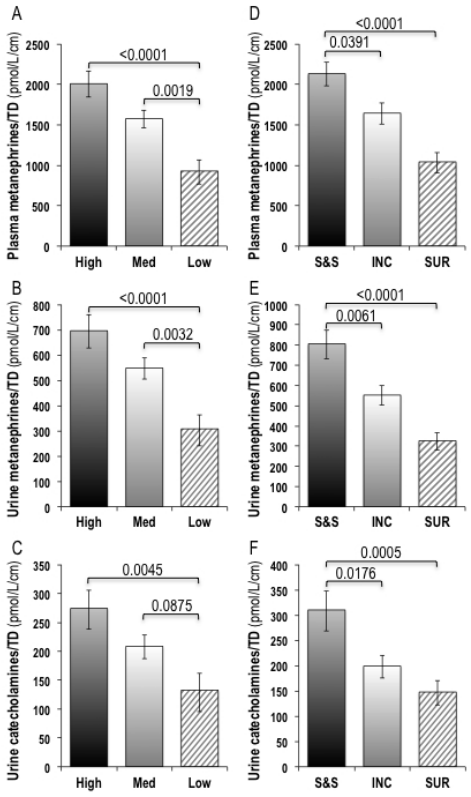

Supplemental Figure 2 / Plasma concentrations of metanephrines (A, D), urinary outputs of metanephrines (B, E) and urinary outputs of catecholamines (C, F) as a function of tumor diameter (TD) in patients with low, medium (MED) and high clinical feature scores (A, B, C) or according to study inclusion criteria (D, E, F). Statistical significance was established by Dunnett's post-hoc tests, which for clinical feature score group comparisons used patients in the low score group as the comparator, whereas for study inclusion group comparisons used the signs and symptoms (S&S) group as the comparator.

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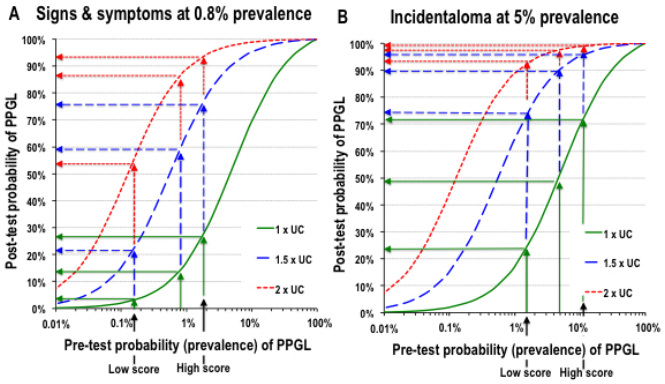

Supplemental figure 3 / Relationships of pre-test versus post-test probability a PPGL according to results for a single elevation of plasma free normetanephrine or metanephrine just above the upper cut-offs (1 x UC, green curves), 50% above upper cut-offs (1.5 x UC, blue curves) or 100% above upper cut-offs (2 x UC, red curves). The two panels serve to illustrate differences in pre- to post-test probabilities among patients with low compared to high clinical feature scores. Panel A illustrates changes in probabilities in a patient tested due to signs and symptoms at a 0.8% pre-test prevalence of disease, whereas panel B illustrates changes in probabilities in a patient tested due to the finding of an incidentaloma at a 5% pre-test prevalence of disease. Relationships are derived from previously published data (1) from the same group of patients that is also available in the public domain through open access.

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